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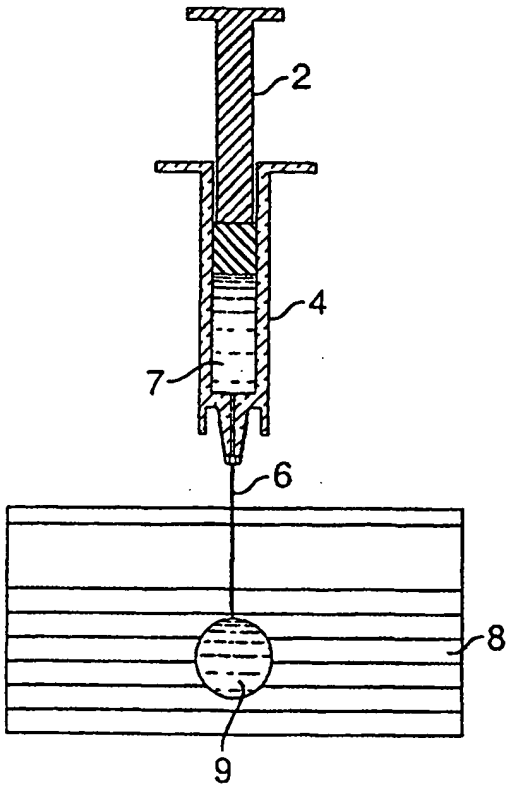
(54) Title: DNA-BASED INTRAMUSCULAR INJECTION SYSTEM FOR HUMANS



(57) Abstract: A method of injecting a DNA-based injectable into a human, using a needle-free injection system (10) is provided. The method includes the steps of first pressuring an injectate (18) within an ampule (12) having a nozzle orifice (16). A peak pressure of approximately 3900-4300 psi adjacent the nozzle, is reached. Then the pressure is immediately reduced to approximately 1200-2100 psi while the injection is continuing. This distributes the DNA-based injectable throughout the muscle tissue and traumatizes the tissue. The pressure is cut-off, normally within 10 ms, to terminate the injection process.

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INTRAMUSCULAR INJECTION SYSTEM FOR INJECTING DNA-BASED INJECTABLES INTO HUMANS

Background of the Invention

Systems for delivering injections into humans have been in use for many years. The most commonly used system is a hypodermic needle attached to an ampule. To perform an injection, the needle is inserted into the tissue to the desired depth and the operator simply depresses a plunger inside the ampule to deliver the injectate. Another method less commonly used is a needle-free injection system. These systems typically consist of a device and an ampule. The device generates the power and the ampule contains the injectate. The ampule typically has a circular opening at its distal end approximately $1/100^{th}$ the size of its inside diameter. The device pushes the fluid out of this opening at speeds fast enough to penetrate the tissue and deposit the injectate. To perform the injection, the operator places the tip of the ampule against the skin of the patient and activates a trigger. For a needle-free injection system, the control of the depth of the injectate is done by the device, not the operator.

Parenteral (a route other than through the gastrointestinal tract) injections are classified according to five well established regions in which the injectate may be deposited. These are: intradermal (ID), subcutaneous (SC), intramuscular (IM), intravenous (IV)/Intraarterial (IA) and intramedullary (IMED). ID injections place the injectate in the skin. SC injections place the injectate in the adipose (fat) tissue. IM injections place the injectate in the muscle. IV/IA injections place the injectate into a vein or artery. Lastly, IMED injections place the injectate in the bone marrow, spinal chord or in the medulla oblongata. Conventional needle and ampule systems can give injections in all five of these regions. Typically, needle-free injection systems are employed only for ID, SC and IM injections. The present invention relates to IM injections.

A needle and ampule system is effective for many types of IM injectables (e.g. MMR and influenza vaccines) because it can assuredly inject a predetermined amount of fluid (typical volumes range from 0.1 to 1.5cc). The needle-free injection system described herein can also administer IM injectables with the same volume range of the same injectables as the needle and ampule system. For either system, the actual injection site on the body can be in many different locations (e.g. the thigh or deltoid).

In the last few years, a substantial effort has been directed into the development of new types of vaccines and therapies. The term "Deoxyribonucleic Acid (DNA)-based injectables" refers to this new class of injectables. DNA is defined as a carrier of genetic information. Vaccines are defined as any preparation intended for active immunological prophylaxis (prevention of a disease). Therapies are defined as the treatment of a disease or disorder by various methods. DNA-based injectables promises to be an exciting new tool for the prevention and treatment of disease.

Briefly, the overall goal of an IM DNA-based injection is to prevent or treat disease. On a cellular level, the goal is to achieve transfection and expression. Transfection is defined as a method of gene transfer utilizing infection of a cell with nucleic acid (as from a retrovirus) resulting in subsequent viral replication in the transfected cell. Expression is defined as the cell's ability to produce the antigen. An antigen is any substance that, as a result of coming into contact with appropriate cells, induces a state of sensitivity and/or immune responsiveness after a latent period (days to weeks) and which reacts in a demonstrable way with antibodies and/or immune cells of the sensitized subject *in vivo* or *in vitro*. Transfection and expression must both occur in order for the injection to be successful. Once transfection and expression have successfully occurred, the genetic "message" contained in the injectate can then be delivered to the immune system. It has been suggested

that in order for an IM DNA-based injection to be effective, the genetic message needs to be delivered to the immune system within a fairly short time after the injection, certainly within several days. It has become recognized that a pooled injection, such as is achieved with a conventional needle and ampule injection, may result in reduced, or complete elimination of, transfection and expression. Needle-free injection systems, other than the one described herein, also have limitations which prevent them from effectively administering IM DNA-based injections (this will be described in more detail later). It is an object of the present invention to develop a needle-free injection system which is particularly suitable for IM DNA-based injectables.

Summary of the Invention

A method of injecting a DNA-based treatment into a human, using a needle-free injection system, is provided. The method includes the following steps: pressurizing an injectate within an ampule having a nozzle to a peak pressure of approximately 3900-4300 psi adjacent the nozzle, immediately reducing the pressure to approximately 1200-2100 psi while the injection is continuing, thereby distributing the DNA-based treatment throughout the muscle tissue and traumatizing the tissue; and cutting off the pressure within 10 ms to terminate the injection process.

Figure List

Fig. 1 is a schematic, side elevation sectional view of an IM DNA-based injection using a needle and ampule injection system, showing injectate being injected into a patient;

Fig. 2 is a schematic, side elevation sectional view of an IM DNA-based injection using the preferred embodiment of the present invention, showing injectate being injected into a patient;

Fig. 3 is a typical pressure profile of a spring powered needle-free injection system;

Fig. 4 is a typical pressure profile of a spring powered needle-free injection system (first 20 milliseconds); and

Fig. 5 is a typical pressure profile of the preferred embodiment of the present invention.

Detailed Description of the Preferred Embodiment

In the preferred embodiment of the present invention, the needle-free injection system used is that described in U.S. Patent No. 5,399,163 or that described in pending U.S. Application Serial No. 08/858,249, both of which are incorporated herein by reference. The preferred embodiment of the present invention envisions a method of injecting a predetermined amount of DNA-based injectate at an IM site in a human. Using the needle-free injection system of the preferred embodiment ensures that the DNA-based injectate is suitably spread throughout the muscle tissue to maximize the likelihood that the injectate will cause the desired immunological response. The goal of the preferred embodiment of the present invention is to deliver DNA-based injectables to an IM site so that the body's immune system is systemically activated to a degree not previously achieved with needle and ampule and other needle-free injection methods.

One way to increase the effectiveness of an IM DNA-based injection is to increase the speed at which the genetic message is delivered to the immune system. This can be accomplished by many methods. Two such methods are: 1) to increase the quantity of cells transfected by depositing all the injectate over as large an area as possible in the target site at a sufficient pressure to ensure transfection; and 2) to administer an IM injection that causes a certain amount of local tissue disruption to occur, which will encourage an immune response. The preferred embodiment of the present invention does increase the speed at which the genetic message is delivered to the immune system.

Figure 1 depicts a conventional injection system including a syringe 2, ampule 4 and needle 6 which is injecting injectate 7 into the many layers of intramuscular region 8 of a patient. The injection is forming a pool or bolus, shown at 9.

Figure 2 shows a schematic cross-section of an IM injection with a DNA-based injectable being directed through the many layers of human muscle tissue. Only a portion of the needle-free injection system 10 is shown in Fig. 2. An ampule 12 with a plunger 14 and injection orifice 16 is depicted injecting injectate 18 into the intramuscular layers 8 of a human patient. It is quite different from the pooling or bolus which results from a conventional ampule and needle injection (see Figure 1). This dispersion pattern deposits the injectate over a large area under sufficient pressure to increase transfection. Second, local tissue disruption is caused in the muscle again by the dispersion pattern. This local tissue disruption is different than the cell transfection described earlier in that transfection occurs at the cellular level and in this context, tissue disruption occurs as separation of, or penetration through, the fibrous tissue surrounding individual muscle cells. Thus, an immune response is activated due to the local tissue disruption.

The proper distribution of injectate through the muscle tissue is dependent upon the injectate being injected at the proper pressure and for the appropriate period of time. As shown in Figure 5, the pressure of the injectate inside the ampule should rapidly rise to a peak pressure of 3900-4300 psi, preferably to about 4100 psi, in less than 5 milliseconds, and preferably in 1 millisecond or less. This phase of the injection is termed the penetration phase. In the penetration phase, the skin, adipose and muscle tissue are penetrated. For the given IM ampule, the peak pressure must be in the range given to ensure penetration of the skin. Injectate pressures below this peak value are not sufficient to consistently pierce the skin layer. Injectate pressures above the

range would penetrate the skin, but are not required and could cause unnecessary pain to the patient. The quick pressure rise is necessary to instantly penetrate to the deepest desired level and avoid any injectate coming back through the tissue, a phenomenon known as "splash-back".

Next the injectate pressure inside the ampule is gradually dropped to about 1200-2500 psi. This phase of the injection, termed the delivery phase, is when the predetermined volume of the IM DNA injectate is delivered to the muscle. It is in this phase that the benefits of the needle-free injection system described herein can be noted. As noted before, the injectate is deposited in the muscle in a unique dispersion pattern. The injectate disperses out over a relatively large area (compared with the needle and ampule injection system). This is basically due to the CO_2 gas power source used in the preferred embodiment of the present invention. The CO_2 gas, coupled with the proper pressure regulating valves and mass flow controls, provides a stable energy source throughout the injection. This translates to a large (between 1200 and 2500 psi) and steady (no significant pressure fluctuations) delivery pressure in the ampule. Another consequence of this large and steady delivery pressure is local tissue disruption which occurs as small separations of, or penetrations through, the fibrous tissue surrounding the individual muscle cells.

Finally, at the end of the injection, the plunger inside the ampule will bottom-out on the ampule itself. This is the only mechanism that stops the injection. Thus, the driving force on the plunger remains high until all the injectate is delivered and because of the plunger-ampule impact, the residual injectate pressure drops to atmospheric pressure in a few milliseconds. The effect of this characteristic is to deliver the entire volume to the desired depth and to prevent the injectate from leaking back through the tissue, a phenomenon known as "leak-back".

Figure 5 depicts a typical pressure profile for a 1/2cc IM DNA-based injection using the preferred embodiment of the present invention. The term pressure profile is defined as a graph of injectate pressure in the ampule vs. time. Data were collected with a state of the art pressure transducer mounted on the ampule so that the sensing element was exposed to the injectate (just upstream of the start of the nozzle) without interfering with the injection. The transducer had a resolution of 0.20 psi and a linearity of 2% full scale. The transducer was connected to a PC based data acquisition system, which consisted of a personal computer, application software, data acquisition board, signal conditioning unit and a power supply. A scan rate of 10,000 samples per second was found to be fast enough to capture the event. This figure shows the injectate pressure in the ampule rising to a peak of about 4000 psi in about 1 millisecond. Immediately following the peak pressure, a 800 psi drop in pressure occurs (down to about 3200 psi) for roughly 1 millisecond. The ampule pressure then returns to its original peak pressure. This phenomenon is probably due to the compliance of the ampule. That is, the ampule was designed to be stiff to easily withstand the pressure, but since its not a perfectly rigid structure, it swells slightly under the large imposed pressure. This swelling means that the diameter of the ampule actually increases slightly, for about 1 millisecond. Thus, some energy is being used to induce this swelling which would otherwise go into pressurizing the fluid. Simultaneously, the ampule plunger transitions from the initial impact to a more of a steady state condition (analogous to the penetration and delivery phase discussed earlier), fluid is expelled out of the small orifice at the distal end of the ampule and the ampule relaxes to its nominal size. This causes the pressure to rebound to its original level. This phenomenon could account for the quick drop and rebound in pressure following the peak pressure. Subsequent pressure fluctuations are much smaller in magnitude (approximately 100 psi) and probably are caused by

the same phenomenon, just on a smaller scale. Although this phenomenon was not part of the design intent, it has no measurable effect on the IM injection and is therefore considered to be tolerable. The curve starts to become truly smooth at about 20 milliseconds and continues to remain so until the end of the injection.

An example of a situation where the pressure fluctuations might be significant for IM DNA-based injections can be found in needle-free injection systems that use a spring (mechanical or gas) as a power source. The normal application for these type of devices are SC injections. Typically, these types of devices use a compressed spring to drive the ampule plunger and give the injection. Figures 3 and 4 show a typical pressure profile for a mechanical spring powered needle-free injection system. The data were acquired with the same system mentioned previously. As with the preferred embodiment of the present invention, the pressure in the ampule rises rapidly to its peak of about 4100 psi in less than 1 millisecond. However, that is where the similarity ends. For the next 9 milliseconds or so, significant pressure oscillations can be seen. At one point, a drop of about 2800 psi occurs. This pressure oscillation translates to a pulsating fluid stream which would have three effects on an attempted IM DNA-based injection: 1) the entire volume would not be deposited at the desired depth, 2) the dispersion pattern would not be optimal and 3) tissue disruption would occur at all tissue layers, rather than just in the target layer (i.e. muscle). Another drawback to using a spring as a power source is that the ampule pressure at the end of the injection is typically very low (roughly 700 psi). This pressure is simply too low to ensure that all the injectate is deposited in the muscle.

Changes and modifications of the present invention can be made without departing from the spirit and scope of the present invention. Such changes and modifications are intended to be covered by the following claims.

I CLAIM:

1. A method of injecting DNA-based injectables into a human, using a needle-free injection system, comprising the steps of:

pressurizing an injectate within an ampule having a nozzle orifice to a peak pressure of approximately 3900-4300 psi adjacent the nozzle orifice, immediately reducing the pressure to approximately 1200-2100 psi while the injection is continuing, thereby distributing the DNA-based treatment throughout the muscle tissue and traumatizing the tissue; and

cutting off the pressure to terminate the injection process.

2. The method of claim 1, wherein the pressure is reduced in a substantially linear manner.

3. The method of claim 1, wherein the pressure is reduced in a generally exponential manner.

4. The method of claims 1, 2 or 3, wherein approximately 0.5 ml of DNA-based treatment is distributed during the injection process.

5. The method of claim 1, wherein the pressure cut-off at the end of the injection occurs in about 10 milliseconds.

6. The method of claim 1, wherein the peak pressure is about 4000-4200 psi, the reduced pressure is about 2000-2200, and the pressure cut-off occurs in about 10 milliseconds.

7. The method of claim 1, wherein the injectate pressure in the ampule has no more than one drop in pressure greater than 500 psi during the first 10 milliseconds of injection.

8. A method of delivering IM DNA-based injectables, using a needle-free injection system, comprising the steps of:

pressurizing an injectate within an ampule having a nozzle orifice to a peak pressure adjacent the nozzle of approximately 3900-4300 psi within 5 milliseconds, thus penetrating the skin, adipose and muscle;

gradually reducing the pressure to approximately 1200-2500 psi, thus distributing the entire volume of the DNA-based injectable over a large area in the muscle tissue, causing improved transfection compared to other injection systems and causing some local tissue disruption, and thereby encouraging an immune response; and

at the end of the injection, abruptly dropping the ampule pressure to atmospheric pressure within 10 milliseconds, thus ensuring the entire volume is delivered to the desired depth and avoiding any injectate leaking back through the tissue.

9. The method of claims 8, wherein the injectate pressure in the ampule, at any point after the peak pressure is achieved in the injection, does not change more than 1000 psi in 1 millisecond or less.

10. The method of claims 8, wherein the injectate pressure in the ampule has no more than one drop in pressure greater than 500 psi during the first 10 milliseconds of the injection.

11. The method of claim 8, wherein the peak pressure is about 4000-4200 psi, the reduced pressure is about 2000-2200, and the pressure cut-off occurs in about 10 milliseconds.

12. The method of claim 8, wherein approximately 0.5 ml of DNA-based injectable is distributed in the muscle tissue during the injection process.

FIG. 1
(Prior Art)

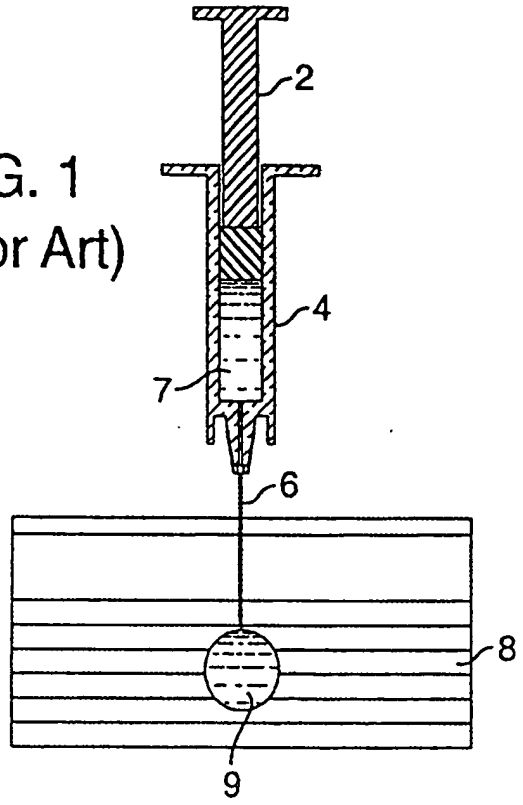


FIG. 2

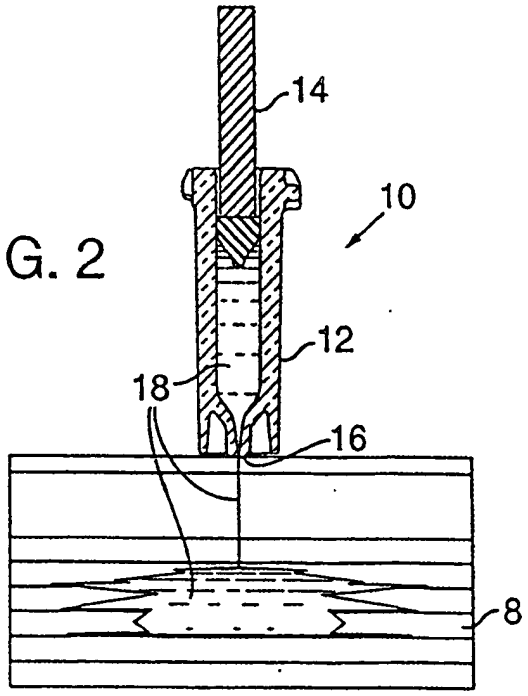


FIG. 3 TYPICAL PRESSURE PROFILE OF A SPRING POWERED NEEDLE-FREE INJECTION DEVICE.

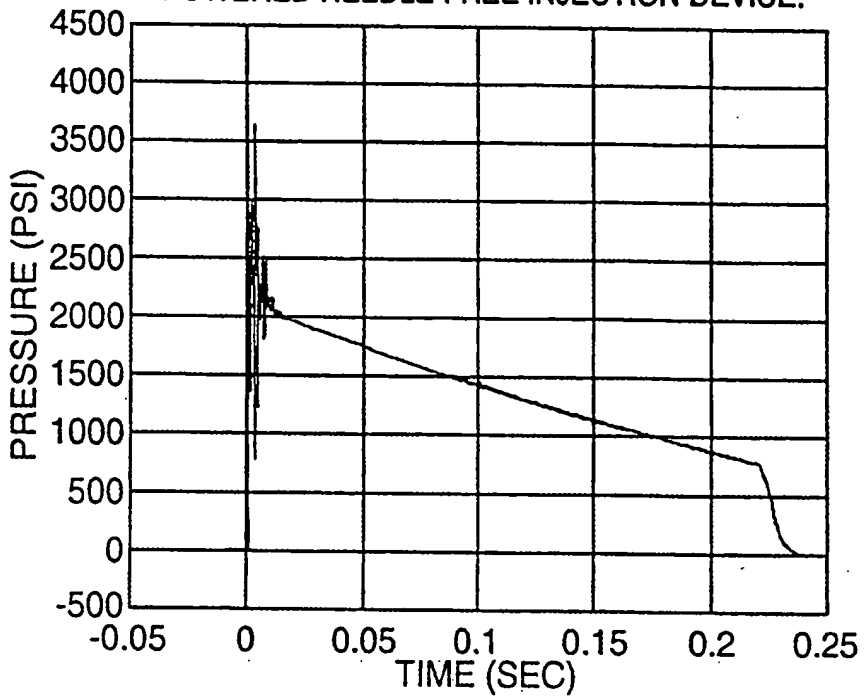


FIG. 4 TYPICAL PRESSURE PROFILE OF A SPRING POWERED NEEDLE-FREE INJECTION SYSTEM (FIRST 20 MILLISECONDS).

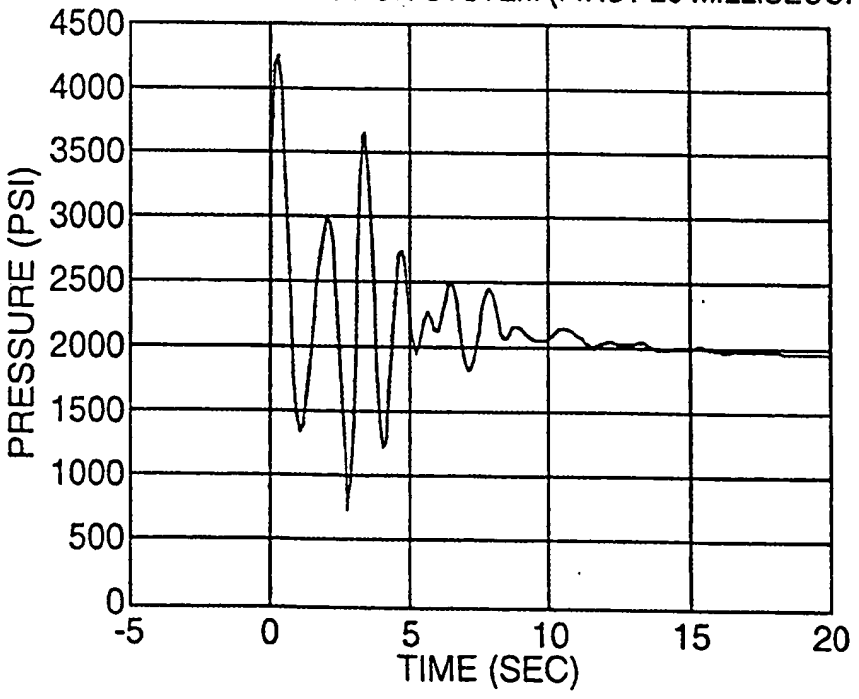
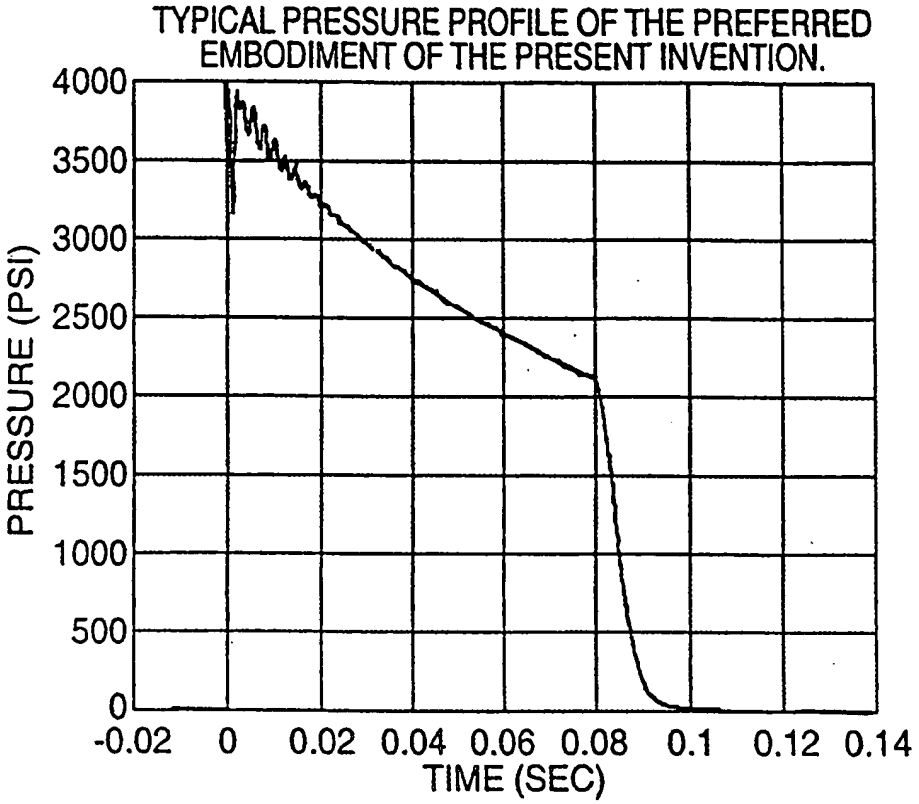


FIG. 5


TYPICAL PRESSURE PROFILE OF THE PREFERRED EMBODIMENT OF THE PRESENT INVENTION.
PRESSURE (PSI)
4000
3500
3000
2500
2000
1500
1000
500
0
-0.02
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
TIME (SEC)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/23001

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61B 19/00
US CL : 128/898

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 128/898, 604/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,399,163 A (PETERSON et al.) 21 March 1995, see entire document.	1-12
Y	Furth, P.A. et al., Gene Transfer into Somatic Tissue by Jet Injection, Analytical Biochemistry, Vol. 205, pages 365-368, 1992.	1-12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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